Subpart D—Mycoplasma

§ 610.30 Test for *Mycoplasma*.

Except as provided otherwise in this subchapter, prior to clarification or filtration in the case of live virus vaccines produced from in vitro living cell cultures, and prior to inactivation in the case of inactivated virus vaccines produced from such living cell cultures, each virus harvest pool and control fluid pool shall be tested for the presence of *Mycoplasma*, as follows:

Samples of the virus for this test shall be stored either (1) between 2° and 8° C. for no longer than 24 hours, or (2) at −20° C. or lower if stored for longer than 24 hours. The test shall be performed on samples of the viral harvest pool and on control fluid pool obtained at the time of viral harvest, as follows: No less than 2.0 ml. of each sample shall be inoculated in evenly distributed amounts over the surface of no less than 10 plates of at least two agar media. No less than 1.0 ml. of sample shall be inoculated into each of four tubes containing 10 ml. of a semisolid broth medium. The media shall be such as have been shown to be capable of detecting known *Mycoplasma* and each test shall include control cultures of at least two known strains of *Mycoplasma*, one of which must be *M. pneumoniae.* One half of the plates and two tubes of broth shall be incubated aerobically at 36° C. ±1° C. and the remaining plates and tubes shall be incubated anaerobically at 36° C. ±1° C. in an environment of 5–10 percent CO_2 in N_2. Aerobic incubation shall be for a period of no less than 14 days and the broth in the two tubes shall be tested after 3 days and 14 days, at which times 0.5 ml. of broth from each of the two tubes shall be combined and subinoculated on to no less than 4 additional plates and incubated aerobically. Anaerobic incubation shall be for no less than 14 days and the broth in the two tubes shall be tested after 3 days and 14 days, at which times 0.5 ml. of broth from each of the two tubes shall be combined and subinoculated onto no less than four additional plates and incubated anaerobically. All inoculated plates shall be incubated for no less than 14 days, at which time observation for growth of *Mycoplasma* shall be made at a magnification of no less than 300×. If the Dienes Methylene Blue-Azure dye or an equivalent staining procedure is used, no less than a one square cm. plug of the agar shall be excised from the inoculated area and examined for the presence of *Mycoplasma.* The presence of the *Mycoplasma* shall be determined by comparison of the growth obtained from the test samples with that of the control cultures, with respect to typical colonial and microscopic morphology. The virus pool is satisfactory for vaccine manufacture if none of the tests on the samples show evidence of the presence of *Mycoplasma.*

Subpart E—Hepatitis Requirements

§ 610.40 Test for hepatitis B surface antigen.

(a) *Test sensitivity.* Each donation of blood, plasma, or serum to be used in preparing a biological product shall be tested for the presence of hepatitis B surface antigen by a method of sufficient sensitivity to detect all sera labeled A, (A), B, (B), and C in the Reference Hepatitis B Surface Antigen Panel distributed by the Center for Biologics Evaluation and Research; except that, in emergency situations, a test method of sufficient sensitivity to detect all sera labeled A, (A), and B in the Reference Hepatitis B Surface Antigen Panel may be used and, in dire emergency situations, blood and blood products may be issued without any HB_8 Ag testing, provided that a test otherwise required by this paragraph is performed as soon as possible after issuance of the blood and blood product.

(b) *Procedures.* Only Antibody to Hepatitis B Surface Antigen licensed under this subchapter shall be used in performing the test and the test method(s) used shall be that for which the antibody product is specifically designed to be effective as recommended by the manufacturer in the package insert. The sample of blood, plasma, or serum to be tested shall have been taken from the donor at the time of donation of that unit. The test need not be performed on the day of the withdrawal of the sample. If the radioimmunoassay method is used, it must be performed in one of the following ways:

(1) The complete test is performed at the collection facility.

(2) The test is performed at the collection facility up to the point of counting the radioactivity of the samples, which counting, thereafter, is performed at another facility by personnel from the collection facility or by personnel from the counting facility.

(3) The complete test is performed by the personnel at an establishment licensed to manufacture blood or blood derivatives under section 351(a) of the